Plastid biogenesis and differentiation

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Abstract

Plastids are crucial to plant functionality and develop from proplastids in meristem cells to generate different plastid forms in different types of plant cells. In addition to the photosynthesis of leaf mesophyll cell chloroplasts, plastids contribute to storage and pigmentation capacities in many different specialised cells as well as contributing essential metabolic pathways within the cell in general. Plastids also have the capacity to interconvert between types according to environmental and molecular signals. Progress in understanding the cell biology and morphological control of different plastid types is considered in the light of modern imaging techniques, which have revealed new aspects of plastid morphology. As well as considering molecular aspects of how plastids control their division, this article discusses also how cell-specific differentiation might be controlled and whether master control genes for plastid biogenesis might be in charge.

1 Introduction

Plastids form a distinct group of organelles in higher and lower plants and are one of the defining characteristics by which plants are different to animals. For many years, most plastid based research focused on the chloroplast and trying to understand the mechanism of photosynthesis and the biochemical interactions of the chloroplast with the cell. With the advent of molecular biology and more recently, a variety of novel imaging techniques, a better understanding of how the chloroplast and other plastid types function within the cell in a truly biological manner is starting to emerge. Even so, the chloroplast remains dominant in providing the bulk of our knowledge about plastid biology. In this article, I consider the structure and morphology of the chloroplast and a range of other plastid types as well as how plastids differentiate and undergo interconversions. Finally, I discuss two fields in plastid biology, which have progressed significantly in recent years, namely plastid division and the biology of stromules.

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2 Proplastids

All plastids within a plant are ultimately derived from those progenitor plastids, which are found in meristem cells called proplastids. These in turn have been derived from the few proplastids, which were present in the zygote and derived potentially from both the maternal egg cell and the paternal pollen grain. However, most Angiosperms have mechanisms to exclude or degrade proplastids in the pollen line and hence the plastids present in the majority of plants are inherited maternally (Mogensen 1996; Corriveau and Coleman 1998; Zhang et al. 2003). In those species in which biparental inheritance occurs, plastids within the zygote constitute a mixed population derived from both parent egg and pollen. However, many factors appear to bias the relative proportion of maternally and paternally-derived plastids and plastid populations in resulting plants can be highly variable with respect to the origins of plastids within them (Mogensen 1996).

Considering the fundamental importance of proplastids to plastid biology, the knowledge of proplastid cell biology and their fine ultrastructure is limited, mostly because of the difficulties with analysing small organelles with no pigment in small regions of dense tissue. Knowledge of the physical appearance of proplastids has been derived largely from electron micrographs (Chaley and Possingham 1981; Akita and Sagisaka 1995; Robertson et al. 1995; Gunning 2004), which show proplastids as small organelles containing limited internal structure that are dispersed throughout the cytoplasm. Most proplastids contain rudimentary pieces of thylakoid membrane, but are unpigmented although those in shoot apical meristems appear to contain more thylakoid in a more organized state than those in the root apical meristem (Gunning 2004). In addition, ingrowths from the inner plastid envelope membrane into the proplastid stroma can also be seen occasionally, as well as ribosomes. Starch grains may be present, especially in proplastids of seeds where starch was laid down in the proplastid during seed development (Gunning 2004). In wheat plumules and potato stolons, starch content of proplastids is variable with some containing significant starch grains and others with no starch. This difference in starch content appears to result from differences in the capacity for starch synthesis since immunogold labelling of the enzyme starch synthase reveals two types of proplastids: those with and those without the enzyme (Akita and Sagisaka 1995).

Estimating proplastid numbers is difficult and to date no studies have definitively counted proplastid populations in meristem cells. However, various studies of shoot meristem cells estimate that they contain 10-20 proplastids per cell (Cran and Possingham 1972; Lyndon and Robertson 1976; Pyke and Leech 1992). Using modern fluorescent protein technology, imaging of proplastids in meristems and during cytokinesis should be feasible, although proplastid dynamics during meristematic cell divisions have yet to be studied in detail. Proplastids with fluorescent marker proteins on board, such as GFP, can be imaged in root meristems (Kohler and Hanson 2000) and those in shoot apical meristems can be observed also (Trynka and Pyke, unpublished), although experiments to determine population

sizes and segregation patterns in different parts of the meristem could prove technically demanding.

Differences in proplastid number according to cell position within the meristem or in organs derived from it may well exist (Lyndon and Robertson 1976), but whether such differences are significant to cellular function are unclear and they may simply reflect differences in proplastid division rate compared to local rates of cell division. Differences in proplastid DNA content and morphology have been shown to exist between cell layers within a meristem, suggesting that tissue-specific characteristics of proplastids within a meristem may be important (Fujie et al. 1994). During the cell divisions of embryogenesis and the cell divisions within meristems, proplastids must divide to ensure continuity within cell lines and to ensure that all cells within the plant contain plastids. Little is known of a distinct mechanism by which a correct proplastid segregation is achieved at cytokinesis (Sheahan et al. 2004) and it would appear that aplastidic daughter cells are prevented simply because proplastids are generally distributed in the cytoplasm, thus ensuring segregation into both daughter cells, but also because they locate more particularly in positions close to the nucleus prior to the onset of mitosis. Positioning in the peri-nuclear cytoplasm during protoplast division is driven by the actin cytoskeleton leading to entrapment of plastids close to the nucleus (Sheahan et al. 2004). Whether a similar process happens during cytokinesis in meristems is unknown. Nuclear mutations, which affect proplastid division and give rise to populations of few, enlarged proplastids in meristematic cells (Robertson et al. 1995) do not result in the appearance of aplastidic cells in meristems, which implies that giant proplastids can still maintain a mechanism by which they segregate correctly. Giant plastids in tomato fruit cells appear able to replicate by a budding/fragmentation mechanism (Forth and Pyke 2006) and therefore it is feasible that the generation of small budded proplastids could ensure correct segregation in meristematic cells containing giant proplastids.

Efforts to study the extent of proplastid metabolism and DNA transcription and translation have been limited but those which have examined proplastid transcription at the tissue level have shown such activity to be low and that the initiation of a differentiation pathway, such as chloroplast differentiation, is necessary to upregulate transcriptional activity (Harak et al. 1995; Mache et al. 1997; Sakai et al. 1998; Baumgartner et al. 1989). Indeed, expression of nuclear genes for proplastid ribosomes is required prior to the expression of those genes, which are plastid encoded. Overall proplastids remain an exasperating organelle, occupying a pivotal place in plastid cell biology but yet about which there is so much still to learn.

3 The morphology and structure of different plastid types

As cells within developing seedlings and developing plant organs differentiate, plastids embark on different patterns of differentiation according to the differentiation pathway that the cell itself takes. Proplastids have the ability to give rise to a

variety of different types of plastid, which form in different types of tissue. Plastids can also interconvert between their different forms in many situations. Thus, for most plastid types, there are two different pathways by which they can arise: directly or by re-differentiation of an existing plastid type. Traditionally, characterisation and naming of different types of mature plastids has largely been based on the types of molecules they store or the types of pigments they accumulate, although this may not necessarily be the best system for plastid taxonomy since often plastids show a mixture of features from different types making precise naming difficult. Although distinct types of plastid differentiation do exist, a better system for their classification could be based on the biochemical and physiological properties or maybe the extent of their proteome or metabolome. Such a system could ease the difficulties by which plastids displaying intermediate phenotypes have to be named. In this chapter, the basic structure and morphology of the major types of differentiated plastids found in higher plants will be consider and subsequently, what is known of the differentiation pathways which give rise to each of the types will be discussed.

3.1 Chloroplast structure and morphology

Chloroplasts are the most prominent form of plastid occurring in all green plant tissues and enable photosynthetic carbon fixation to occur in addition to a variety of other biochemical processes central to cellular metabolism. Like all plastids, they are bounded by a double plastid envelope membrane, which acts as a major control point for chloroplast import and export as well as being a major site for biochemical synthesis (Joyard et al. 1998). Chloroplasts in leaf mesophyll cells are typically ellipsoidal in shape but with defined poles, a feature that is crucial to their division. However, chloroplasts can also be highly pleiomorphic and can take up irregular morphologies in different cell types. Indeed, the potential plasticity in plastid shape has become clear in recent years with the analysis of giant plastids, which occur when plastid division is perturbed. In these giant plastids, which are up to 50-fold larger than normal chloroplasts, the morphology is highly irregular (Pyke et al. 1994) yet apparently stable when osmotically challenged (Pyke 2006) suggesting that a mechanism exists which controls and exerts stability on plastid morphology. A suggestion that an FtsZ-based internal plastoskeleton might function in controlling plastid morphology (Reski 2002) needs further experimentation since most of the FtsZ molecules within the plastid appear to be involved in division rather than morphological control. The recent discovery of mechanosensory proteins within the plastid envelope (Haswell and Meyerowitz 2006) showed that perturbation of such proteins by mutation affects plastid morphology, implying that tension monitoring in the plastid envelope somehow plays a role in morphological control.

A major structural component, which typifies the chloroplast, is the extensive thylakoid membrane system, which extends throughout the body of the chloroplast and dominates its internal architecture. Thylakoid membranes are the site of photosynthetic electron transport and ATP synthesis and delimit a distinct compart-

ment within the chloroplasts: the thylakoid lumen. Thylakoids are composed of lamellae, which are arranged into a highly complex system of stacked lamellae called grana interconnected by single lamellae called stromal lamellae.

Models for thylakoid membrane structure have been developed largely from analysis of electron micrographs of sectioned chloroplasts, a system that is fraught with difficulty in interpretation in generating three-dimensional models from two-dimensional images. Three different models have been proposed (Arvidsson and Sundby 1999; Mustardy and Garab 2003; Shimoni et al. 2005) but differ in their conclusions, although all show the highly complex nature of thylakoid membrane arrangement within the grana. The model of Mustardy and Garab (2003) shows the grana as fused stacks of membrane which look like fan blades, with stromal lamellae joining stacks together at alternating levels within the stack, and the whole structure forming a right handed helix. The reason for this complex thylakoid membrane morphology is to provide a large surface within the plastid on which light capture by chlorophyll and electron transport can occur. Consequently, the area of thylakoid membrane within a mature plastid is large and much greater than simple invaginations from the plastid envelope membrane.

Surprisingly, the mechanisms by which the construction of the thylakoid membrane system is initiated, synthesised in large amounts and then built into a complex three-dimensional architecture is poorly understood. Electron micrographs showing invaginations of the inner plastid envelope into the stroma gave credence to the hypothesis that thylakoid membrane is derived, at least initially, from such invaginations as proplastids differentiate into chloroplasts. Proplastids usually contain small amounts of thylakoid membrane and the extensive biogenesis of more thylakoid membrane may simply involve building off of extant membrane. However, recent studies have clearly shown that both chloroplasts and proplastids contain vesicles within the stroma (Westphal et al. 2003; Gunning 2004) and that a vesicle trafficking system occurs in plastids primarily between the plastid envelope and the stroma (Westphal et al. 2003). Vesicles are budded from the inner plastid envelope and accumulate close to the inner membrane, particularly when fusion processing at the thylakoid membrane is curtailed by low temperature (Morre et al. 1991). The main purpose of plastid vesicle transport is probably that of providing galactolipids, which are synthesised in the plastid envelope membranes (Joyard et al. 1998), for continued synthesis of thylakoid membrane, although they could also deliver hydrophobic proteins, which reside in the thylakoid membrane. Plastid vesicle trafficking appears to utilize several homologous components of the cytosolic ER Golgi trafficking system, encoded by nuclear genes, in that the chloroplast contains both ARF1 and Sar1 GTPases (Andersson and Sandelius 2004), which are involved in vesicle assembly. In addition, the chloroplast also contains dynamin (Park et al. 1998) and proteins required for vesicle fusion (Hugeney et al. 1995). Two other nuclear-encoded proteins involved in the vesicle directed thylakoid biogenesis are VIPP1 (Kroll et al. 2001) and Thf1 (Wang et al. 2004). Mutations in either gene result in abolition of vesicles and perturbed synthesis of the thylakoid membrane. VIPP1 forms a high molecular weight complex on the inner envelope membrane, which could conceivably be involved in vesicle production (Aseeva et al. 2004). An intriguing problem for the future will be to

understand how vesicle directed thylakoid synthesis is controlled to facilitate the construction of thylakoid architecture and biogenesis of the correct three-dimensional arrangement of the thylakoid membrane network. FZL is a dynamin-related membrane remodelling protein and is located inside the chloroplast in punctate foci on the plastid envelope and on the thylakoid membrane (Gao et al. 2006). Perturbation of this protein results in altered thylakoid morphology and changes in patterns of granal stacking suggesting that it plays an important role in thylakoid organisation and possibly in the dynamic continuum of membrane synthesis between the plastid envelope and the thylakoid.

During chloroplast development there is a significant increase in size of the plastid organelle from proplastid to mature chloroplast. There is also significant variation in mature chloroplast size in different cell types and also within the population of chloroplasts within individual leaf mesophyll cells. An important question yet to be addressed is what mechanisms control chloroplast size? Within a population of chloroplasts in a leaf mesophyll cell there is a trade-off between plastid density and size such that permutations of more small ones or fewer larger ones can be observed in cells of differing sizes and in different species where average leaf mesophyll cell size varies (Ellis and Leech 1983; Pyke 1999). However, the expansion process by which chloroplasts increase the surface area of their envelope membrane and the extent of the stroma and the thylakoid membrane must have a control system which shuts down further expansion at maturity. Conceivably a mechanosensing mechanism (Haswell and Meyerowitz 2006) could achieve this so that as chloroplasts become more densely packed and start squashing each other, as happens in leaf mesophyll cells, mechanosensing feedback shuts down further plastid replication and plastid expansion.

3.2 Amyloplast structure and morphology

All chloroplasts seem to have the ability to accumulate starch grains within the stroma as a transient store of photosynthetic assimilate. Normally these starch grains are degraded through the dark part of the photoperiod and the products exported. Amyloplasts, however, are a plastid type in which starch accumulation is long term and are mostly found in storage tissues such as tubers and seed endosperm where they are highly abundant. All plant starch is synthesised in the plastid and produced either directly from photosynthate, as occurs in leaf plastids or indirectly from photosynthate transported to heterotrophic tissues within the plant. The latter process occurs in amyloplasts, which are the dominant organelle in storage tissues and are of great agricultural and economic significance since 75% of the energy contained in the average human diet is derived from starch (Duffus 1984). Starch is an insoluble, complex, semi-crystalline polymer of glucose synthesised in amyloplasts by the polymerisation of ADP glucose, producing highly branched amylopectin and relatively unbranched amylose in proportions of 70:30% (Smith et al. 1997). Starch is present within amyloplasts as grains, which have a distinct structure consisting of a series of concentric rings alternating between semi-crystalline and amorphous zones. These zones are a result of differ-

ences in organisation of the amylopectin chains (Smith et al. 1997). Two different size classes of starch grains are present in endosperm amyloplasts; the A-type, of up to 45 μm in diameter, and the smaller B-type of up to 10 μm in diameter. The ratio of these two types is variable and under environmental control but has a major effect on the processing qualities of the resultant starch in the food industry (Langeveld et al. 2000).

Amyloplasts most often form from proplastids during the early development of storage organs such as tubers or seed endosperm. In red winter wheat, proplastids are present within the coencytic endosperm but when cellularisation is complete, starch deposition commences and amyloplasts are formed (Bechtel and Wilson 2003). Some understanding of the way in which amyloplast differentiation is controlled has come mostly from exploiting cell cultures, in which amyloplast differentiation can be induced by adding phytohormones. Tobacco BY-2 cells grown in the dark are undifferentiated and contain proplastids. The presence of auxin causes these cells to proliferate. When auxin is replaced with benzyladenine, rates of cell division decline and proplastids accumulate starch and form amyloplasts (Sakai et al. 1992, 1999).

Amyloplasts are also present in a specialised cell type in the root tip; the collumella cells. Collumella cells form the gravisensing system in roots enabling gravitropic responses in growth in relation to the gravity vector. The sinking of amyloplasts, called statoliths in these cells, in the cytoplasm under the influence of gravity is thought to initiate a signal transduction pathway involving auxin redistribution (Swarup et al. 2005), which results in differential cell expansion and downward growth in a positively gravitropic manner. The reverse happens in stems of shoots where statoliths are present in a sheath of cells around the vasculature and cause upward growth of shoots in negatively gravitropic manner (Yamamoto et al. 2002). How these amyloplasts form specifically in these two cell types whilst cells around them contain different plastid types is unclear but undoubtedly the control is more complex than a simple change in the type of phytohormone as suggested by differentiation in cell cultures.

Since chloroplasts in many plant species accumulate significant amounts of starch during the light period, it may be pertinent to consider how these starch-laden chloroplasts differ from amyloplasts where starch storage is more long term. Transient starch in chloroplasts has a lower amylose content, forms as flattened plate-like structures rather than the more spherical grains of amyloplasts and does not possess the growth rings of amyloplast starch (A. Smith, personal communication). In some species such a tobacco and cotton, starch breakdown in older leaves is not complete by dawn and starch accumulates in these older leaf cells, taking on some properties of long-term storage starch. Conversely some species synthesise very little starch in the chloroplast at all during the light period and export their photosynthate and synthesise sucrose in the cytosol (Zeeman et al. 2004).

3.3 Chromoplast structure and morphology

During the evolution of higher plants, a necessity arose in that plants needed to attract insects and mammals to them in order to facilitate flower pollination and to aid in the dispersal of seeds within fleshy fruits. In order for higher plants to be prominent visually within the flora, the development of brightly coloured structures occurred, primarily in the petals of flowers and in the tissues of fleshy fruits. The accumulation of pigments within the plastids in these tissues led to the formation of a distinct type of plastid, namely the chromoplast. Most of the pigments that are laid down in chromoplasts are carotenoids, which are synthesised from the C_{40} molecule phytoene, and constitute several different types, namely carotenes, lycopene, lutein, violaxanthin, and neoxanthin (Camara et al. 1995; Cunningham and Gantt 1998; Bramley 2002). These classes of molecules are not the sole preserve of chromoplasts, since several are commonly found on thylakoid membranes in the chloroplast where they function as accessory pigments in light capture and energy dissipation. In addition, the carotenoid- related pigment astaxanthin is the basis of the red-pink colouration in several animals including flamingo, lobster, and shrimp (Armstrong and Hearst 1996). Other types of soluble pigments, which are found in the cell's vacuole, also contribute to colouration of plant parts and in many cases a mixture of pigment types is present (Kay et al. 1981; Weston and Pyke 1999).

Detailed structural analysis of chromoplasts in different tissues and species shows great heterogeneity in their structure, which probably reflects differences in the profile of coloured pigments present. There have been efforts to categorise chromoplasts into distinct types according to the types of storage structures present within the chromoplasts, i.e. globular, membranous, or crystalline (Thomson and Whatley 1980; Camara et al. 1995). Although laudable, such a classification system can be difficult to apply to the vast range of chromoplast types found throughout nature in different tissues in different plant species. Knowledge of chromoplast biogenesis has been gained largely from a detailed study of agriculturally important fleshy fruits, primarily in tomato (*Lycopersicon esculentum*) (Fraser et al. 1994; Camara et al. 1995; Cunningham and Gantt 1998), and bell pepper (Hugueny et al. 1995a). During the formation of pigmented chromoplasts from green chloroplasts in unripe fleshy fruit, a controlled breakdown of chlorophyll and the thylakoid membrane occurs concurrent with a significant increase in carotenoid pigment biosynthesis. Increased expression of the ELIP gene is associated with the chloroplast to chromoplast transition and may play a role in the regulated breakdown of the extensive thylakoid membranes of the chloroplast (Bruno and Wetzel 2004). Associated with increased carotenoid biosynthesis is the upregulated expression of several nuclear genes, which are required for chromoplast differentiation (Lawrence et al. 1993, 1997; Summer and Cline 1999). Plastid DNA appears to play a minor role in chromoplast differentiation and there is increased methylation of plastid DNA in chromoplasts (Kobayashi et al. 1990). Exactly how a chromoplast differentiation pathway is initiated in green chloroplasts in ripening fruit is unclear, even though a significant amount is known about the basic biochemistry and molecular biology of fruit ripening and the role of the

hormone ethylene (Alexander and Grierson 2002). It could be argued that the chromoplast is no more than a bag into which carotenoid pigment is loaded and indeed increased transcription in a variety of carotenoid biosynthetic enzymes is a key phase in chromoplast biogenesis. Increases in enzyme activity of phytoene synthase and phytoene desaturase (Fraser et al. 1994), 1-deoxy-D-xylulose 5-phosphate synthase (Lois et al. 2000) and a plastid terminal oxidase associated with phytoene desaturation (Josse et al. 2000) are observed as chloroplast differentiate into chromoplasts. There are also increases in other proteins not associated with carotenoid metabolism and which could be best viewed as chromoplast specific differentiation genes. These include enzymes in response to oxidative stress (Livne and Gepstein 1988; Romer et al. 1992), and carotenoid sequestration proteins including fibrillin (Vishnevetsky et al. 1999).

Surprisingly the cell biology of chromoplast differentiation has been poorly described and until recently was dependent upon electron microscopy descriptions of their structure (Harris and Spurr 1969a, 1969b; Thomson and Whatley 1980; Bathgate et al. 1985). Internally, chromoplast structure appears dependant on which type of carotenoids are sequestered within them since the internal architecture is highly variable and can consist of either plastoglobules of pigment, crystalline structures of carotenoids, microfibrillar structures with sequestered carotenoids, extensive internal membranous structures or a mixture of these. Gunning (2004) shows particularly beautiful colour images of red and yellow chromoplasts in a variety of petals and fruits.

In recent years, the exploitation of green fluorescent proteins targeted to the plastid compartment has enabled chromoplasts to be observed within the whole cell and some aspects of their cell biology have been revealed, primarily in the ripening fruits of tomato (Fig. 1). In the light microscope, these red tomato chromoplasts appear as small heterogeneously shaped organelles with little clear structure. The production of thin membranous tubules from the chromoplasts called stromules, a feature of plastids in general, has been well studied and will be discussed in Section 6. Occasionally, the membrane of tomato chromoplasts is distorted by long thin crystals of lycopene (Pyke and Howells 2002). Mature pericarp cells in the fleshy part of the ripe tomato fruit are large and may contain up to 2000 red pigmented chromoplasts, which are generated from populations of dividing chloroplasts, that accumulate during the green phase of fruit development. During the differentiation of chromoplasts from chloroplasts, a heterogeneous array of small bodies within the cell can be observed, some of which appear to be broken pieces of stromule or even vesicles which appear to bud from the chloroplast body and are revealed by the GFP they carry (Waters et al. 2004; Forth and Pyke 2006). Thus, two different processes could give rise to large populations of differentiated chromoplasts within the cell and gives support to the idea that chromoplasts are little more than storage sacs with high levels of carotenoid biosynthetic enzyme activity.

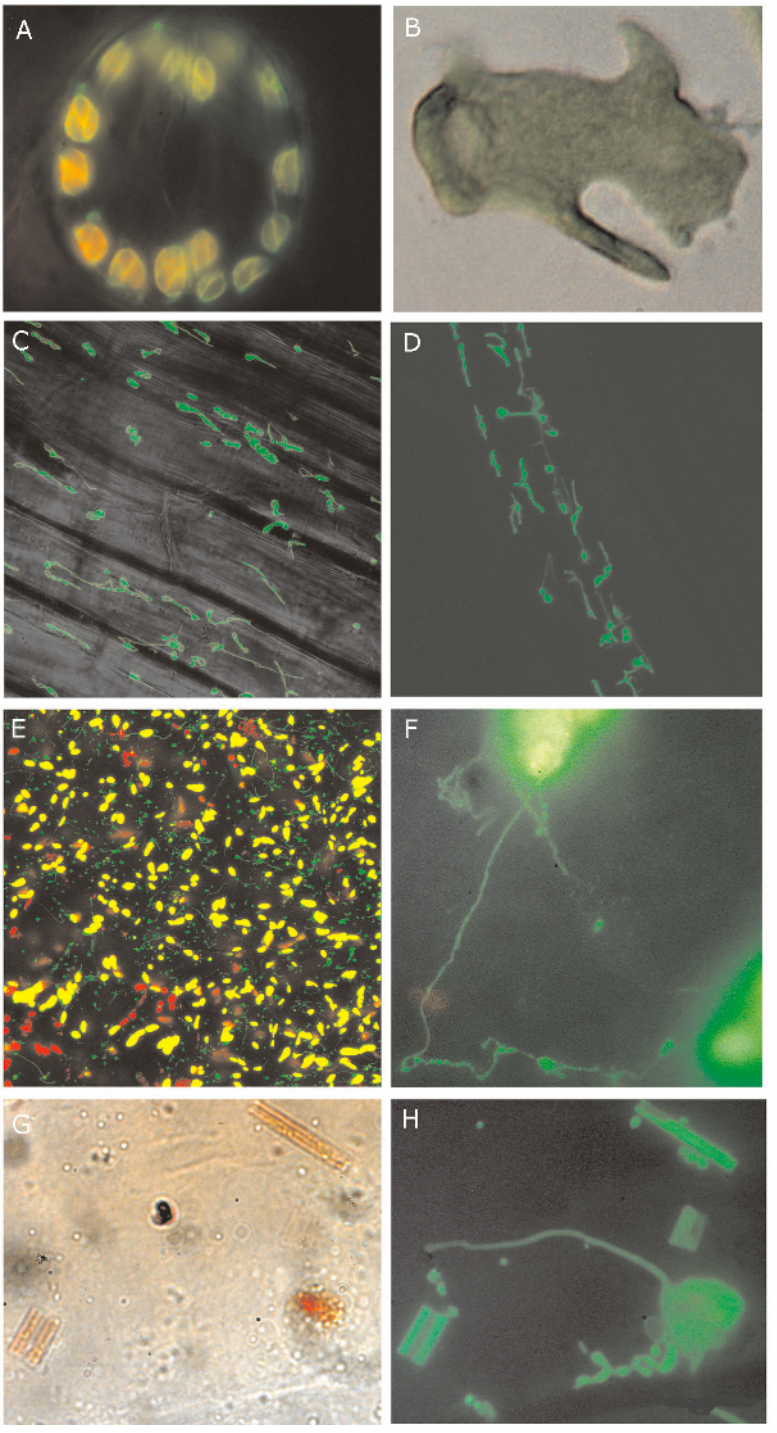
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Fig. 1 (overleaf). Variation in plastid morphology. (A) Chloroplasts in a leaf stomatal guard cells containing GFP, which fluoresces green on a background of red chlorophyll fluorescence. These chloroplasts have a conventional chloroplast morphology and have only small stromule protrusions. (B) Isolated giant mesophyll cell chloroplasts from *Arabidopsis* leaves expressing antisense copies of the FtsZ plastid division protein. These chloroplasts are highly variable in shape but maintain their complex morphologies when isolated from the cell. (C) Plastids in the hypocotyl cells of a tobacco seedling illuminated by targeting green fluorescent protein to the plastids. These plastids show extensive stromules and complex looping. (D) Plastids in the epidermal cell of an *Arabidopsis* root illuminated by targeting green fluorescent protein to the plastids. These plastids are highly variable in morphology, at the most extreme showing thin stromules. (E) Image of a pericarp cell in a tomato fruit at the onset of chromoplast differentiation from chloroplasts. Green fluorescent protein has been targeted to the plastids. Yellow plastid bodies exhibit both green fluorescent protein fluorescence as well as red chlorophyll fluorescence. Some red plastid bodies contain little GFP. A large number of plastid-derived structures, which contain bright green GFP are visible, both as stromules and distinct vesicle-like structures which appear to bud off from the main plastid bodies and lack chlorophyll. (F) Two chromoplasts in a tomato pericarp cell, which contain extensive GFP in the main chromoplasts bodies and are connected by two long thin stromules which show significant beading. (G and H) The cytoplasm of a pericarp cell from a ripe tomato view with brightfield (G) and with GFP fluorescence targeted to the plastid. A stromule emanating from a plastid body is obvious as are crystals of lycopene, which contain GFP and presumably are surrounded by a chromoplast membrane.

3.4 Leucoplasts and root plastids

Leucoplast is the name given to a general group of plastids, which lack any pigment and are often referred to as non-green plastids. Leucoplasts are very widely distributed in different plant tissues and have a wide range of morphologies and content, the latter being primarily a variation in the type of storage molecules that they accumulate. In fact amyloplasts could be considered a form of leucoplast that has specialised in storing starch. Whilst they are widespread in plant tissues, the general cell biology of leucoplasts has not been extensively investigated although many aspects of their biochemistry have been examined under the umbrella of non-green plastids (Emes and Neuhaus 1997; Eastmond et al. 1997). Leucoplasts can be isolated in clean populations from seed endosperm tissues and their biochemical characteristics examined (Negm et al. 1995).

A major class of leucoplasts are those found in different types of root cells of and often referred to as root plastids. These undoubtedly play a central role in root metabolism and function and many aspects of their biochemistry and metabolism have been described in detail (Emes and Neuhaus 1997; Debnam and Emes 1999; Fox et al. 2001). Early work examining electron micrographs of root plastids showed that proplastids in cells leaving the root apical meristem lose any thylakoid-like structures and pass through a transient phase of starch accumulation before becoming highly amoeboid in shape and then finally discoid with significant amounts of pregranal structures (Whatley 1983).

As with chromoplasts, the targeting of GFP to the plastid compartment has allowed the direct observation of root plastids in living tissue and they appear highly variable in morphology and exhibit many structures, which are reminiscent of stromules (Fig. 1). Indeed it is difficult to separate the presence of stromules on these plastids with variation in their morphology to the extent that stromules might be regarded as the most extreme characteristic of their morphological form. The cellular distribution of root plastids and leucoplasts in other tissues appears to be directed and non-random, since targeting of GFP to the plastid compartment reveals that leucoplasts commonly associate with the nucleus in an intimate manner, in that they surround the nucleus and are even found to lie within grooves in the nuclear membrane (Kwok and Hanson 2004). Such an association would seem to facilitate efficient signalling between plastid and nucleus and may also be a strategy for ensuring correct plastid segregation at cytokinesis (see Section 2).

Recent studies have revealed a novel role for plastids in directing the interaction of the root cells with symbiotic fungi and bacteria. Firstly, extensive plastid stromule networks develop in cells in arbscules where they interact with the fungal surface (Fester et al. 2001; Hans et al. 2004). Secondly, there is a major upregulation in plastid metabolic activity in these cells, as shown by transcript and metabolite profiling which provides a variety of metabolites which are central to the symbiotic interaction with the invading symbiont and the synthesis of the symbiotic structures such as the peri-arbuscular membrane (Lohse et al. 2005). Moreover two plastid membrane proteins, CASTOR and POLLUX, are crucial to the microbial admission into root cells, which forms the very first stage of the symbiotic relationship (Imaizumi-Anraku et al. 2005). Thus, it appears that a pre-existing endosymbiont in root cells, the plastid, and aids the integration of free-living soil bacteria into a symbiotic relationship with plants.

3.5 Other types of storage plastids

In addition to coloured pigments and starch, plastids are capable of accumulating other types of storage material. These can include lipids, which accumulate in elaioplasts and proteins, which accumulate in proteinoplasts. In both cases such plastids are found often in specialised cells within complex tissues. For instance, elaioplasts are commonly formed in the tapetal cells of the anther where they accumulate large amounts of neutral esters (Ting et al. 1998), which are released by elaioplast breakdown and contribute to the lipid component of the pollen wall (Clement and Pacini 2001). Storage lipids in plastids occur in structures called plastoglobules, which are commonly found in all plastid types. It is the extent of plastoglobule production, which essentially defines an elaioplast from any other plastid type, since elaioplasts are generally packed full of plastoglobuli. A recent proteome analysis of plastoglobuli reveals they contain several proteins involved in metabolism of isoprenoid derived molecules as well as fibrillins, which form a protein coat around the exterior of the plastoglobulus preventing coalescence (Ytterberg et al. 2006). This suggests that plastoglobuli have a metabolic role in the plastid rather than simply being a storage sac. It is unclear whether such a pro-

teome and metabolome profile varies significantly between plastoglobuli in elaioplasts and those plastoglobuli, which appear less abundantly in other plastid types such as chloroplasts.

4 The control of plastid differentiation

The type of plastid present in a given type of cell is dictated by the nature of that cell type. Exactly how this developmental system is controlled by the host cell is largely unknown. It is normally assumed that the differentiation of proplastids into mature chloroplasts is the default pathway of plastid development, occurring in much of the above ground tissues in most plants. The leaf is a good organ in which to consider variations in tissue-specific chloroplast development. The fine tuning of this developmental process is significant since different cell types in a leaf all contain chloroplasts but these chloroplasts vary significantly in size, the extent of chlorophyll accumulation and membrane synthesis as well as large differences in their abundance within the cell. The most authentic development occurs in palisade and spongy mesophyll cells, where chloroplasts pack the cytoplasm and individual chloroplasts are fully photosynthetic with extensive thylakoid membrane and high levels of chlorophyll. In all other types of leaf cells, chloroplast development is less extensive and could be considered repressed. Although all other cell types in the leaf such as bundle sheath cells, epidermal pavement cells, vascular tissue, stomata, and hair cells have chlorophyll-containing chloroplast, the chloroplasts are all smaller, less well developed, and less abundant per cell. The implication is that a cell-specific repressive signal perturbs normal chloroplast development in these cells, resulting in poorly developed chloroplasts. Although photosynthetically compromised, these chloroplasts perform a crucial role in cellular metabolism in these different cell types and without them cellular function would be highly compromised.

A fundamental point of control in chloroplast differentiation is the presence of light, which initiates a complex chain of events inducing gene expression and protein synthesis, which in turn generates the proteome and the resulting metabolome of the mature chloroplast. A tight interaction between the developing chloroplast and cellular differentiation is crucial during this stage and a key part of this is a retrograde signalling pathway from the developing chloroplast back to the nucleus, which induces patterns of expression for genes, which encode plastid-destined proteins. Details of these molecular processes have been discussed extensively in recent times (Moller 2004; Lopez-Juez and Pyke 2005; Lopez-Juez 2007) and are also considered in other chapters in this book and will be considered only briefly here.

The big question remains as to what are the major control genes, which enable chloroplast differentiation to occur in a light–induced manner in mesophyll cells but not to the same extent, for instance, in neighbouring epidermal cells. Mutant screens for chloroplast biogenesis genes have identified a vast array of lines, mutant in genes which are critical for normal chloroplast function and which result in

pale compromised seedlings. Many studies have shown that perturbation of genes which have basic functions in the chloroplast, result in pale compromised chloroplasts; for instance, mutation of the RpoTp RNA polymerase (Hricova et al. 2006) or components of the Clp protease core (Rudella et al. 2006). Sifting out from such collections, mutants that represent the major control genes in this system is very difficult, although directed efforts in this direction are being made (Gutierrez-Nava et al. 2004). Indeed one might forecast that mutation in a global master switch for plastid development would be embryo lethal and therefore unlikely to figure in screens for pale mutants. Lopez-Juez (2007) considers the possibility of global master switches, which facilitate chloroplast development from proplastids. Several candidates are possible although none have compelling evidence to merit them being in complete charge. Maybe the most likely candidates at present are *GLK* genes, which encode transcription factors and appear to be conserved in all land plants but not in single-celled photosynthetic organisms. Maize and *Arabidopsis* contain two *GLK* genes and when both are mutated, chloroplast development and thylakoid biogenesis is dramatically perturbed (Fitter et al. 2002; Yasamura et al. 2005). Intriguingly, *GLK* genes are not sufficient to overcome the general repression of chloroplast development in non-green tissues, as *GLK* overexpressing plants fail to develop green roots, for example, and thus function only in the correct developmental context.

Progress in understanding how plastids develop and the precise differences between differentiated plastid states will likely come from proteomic analyses of distinct cell types and the plastids within them (Kleffmann et al. 2006). Such technology has the potential to pinpoint subtle differences between plastids that currently are unknown. For instance, differences between chloroplasts in bundle sheath and mesophyll cells in leaves as highlighted by proteomic analysis, reveals subtle differences in addition to the basic known differences in photosynthetic metabolism (Majeran et al. 2005). It seems likely that progress using such strategies may well reveal that even the chloroplast actually represents a collection of subtly different organelles reflecting their precise development in different types of cell.

4.1 Plastid interconversions

Although chloroplast differentiation from proplastids, as directed by light, appears the central tenet of plastid biogenesis, there are many examples in which plastids can redifferentiate from pre-existing plastid types and form a different type of plastid (Fig. 2). Such interconversions are controlled by cellular developmental processes as well as environmental or hormonal signals and demonstrate an extreme plasticity in the plastid's functionality within the cell. Although several of these interconversion processes have been described, little is known of the exact molecular control of such redifferentiation processes. The best studied interconversion is that of chloroplasts redifferentiating into chromoplasts during fruit ripening, as discussed previously in this article. In tomatoes and peppers, the chromoplast differentiation pathway has a clear endpoint in mature ripe fruit, but in other systems such as orange citrus fruit ripening and maturation of pumpkins, the

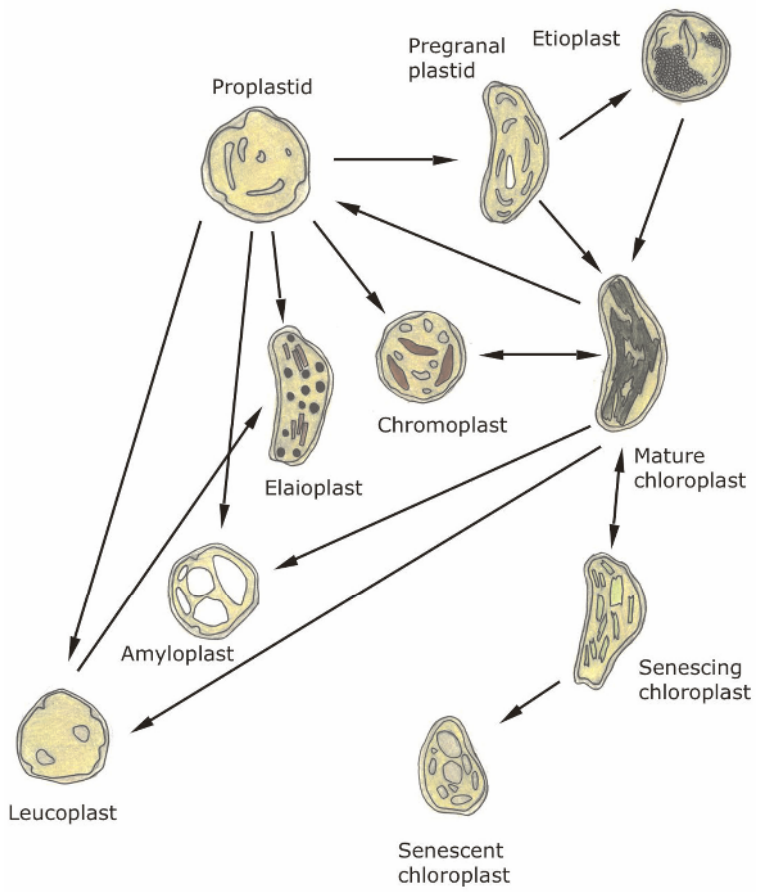

Fig. 2. A general scheme for interconversions of plastid types in different plant tissues. Although various routes for plastid interconversions are arrowed, it is likely that in various specific instances in different tissues, the majority of plastid types can interconvert to a different type.

orange chromoplasts are capable of reverting back to green chloroplasts. Application of the hormone gibberellin further promotes this process in oranges (Thomson et al. 1967). Another plastid redifferentiation pathway, which has major agronomic consequences, is the formation of chloroplasts from amyloplasts in the tissues of potato tubers as a result of illumination (Virgin and Sundquist 1992; Ljubicic et al. 1998). Although significant efforts are made to prevent such tuber greening during potato storage, the reason why this amyoplast-chloroplast interconversion is enabled in potato storage cells but prevented in other amyloplasts containing tissues, such as endosperm, is unclear. In reality, plastid interconversion is a common process during the development of complex tissues. For instance during the development of the anther, there is a complex pattern of interconver-

sion between proplastids, amyloplasts, chromoplasts, chloroplasts, leucoplasts, and elaioplasts which varies in its nature according to the specific tissue type within the anther (Clement and Pacini 2001). In the face of such complex interactions, it could be more prudent to consider that no plastid differentiation pathways are terminal and that all plastids have the ability to change between different states according to the precise information derived from the cell.

5 Plastid division

The fact that plastids can divide as distinct organelles within the cytoplasm of the eukaryotic plant cell was confirmed by several studies in the late 1960s in which populations of plastids were counted and changes in their population size were established in correlation with cell expansion in developing leaves (see Pyke 1997). These studies clearly showed that there were two different points in plastid development where division takes place. Firstly in dividing cells in the meristem, proplastids are required to divide in order to maintain their lineage in newly divided cells. Without such a division, proplastids would likely be lost and aplastidic cells would be generated. Secondly, during the expansion phase of leaves, mesophyll cells increase in volume and the young chloroplasts divide during this period in order to maintain a population in an ever-enlarging cell. The final outcome of this process is mature mesophyll cells containing large populations of individual chloroplasts. The actual number of chloroplasts present is mainly related to the size of the cell, a relationship that extends across different species. In mature leaves in most species, the mesophyll cells contain between 50 and 200 chloroplasts. It is normally assumed that the basic mechanism by which plastids divide is the same for proplastids and for young chloroplasts although the control factors for these two processes are likely to differ. Cells in other green tissues in plants also accumulate chloroplasts in a similar manner although the end point of plastid population size and the size of individual plastids in different tissues and cell types varies greatly.

The plastid division process involves the constriction of the plastid centrally, which eventually leads to a pinching of the envelope membrane and fusion producing two separate daughter plastids, a process termed binary fission.

Progress in understanding the molecular basis of the plastid division machinery has been significant in the last 15 years due to approaches on two fronts. Firstly mutants of *Arabidopsis* were identified in which chloroplast numbers in leaf cells were altered significantly and secondly, genes involved in prokaryotic cell division were discovered in plant genomes and shown to function in plastid division (Pyke and Leech 1994; Pyke 1999). These two approaches have revealed many nuclear genes and their associated proteins and have enabled working models to be developed of how plastids divide (Aldridge et al. 2005). Central to the division process is the formation of a constriction ring composed primarily of FtsZ proteins that resides on the inside of the plastid envelope in the stroma (Osteryoung and Vierling 1995; Osteryoung et al. 1998; Vitha et al. 2001; McAndrew et al. 2001;

Kuroiwa et al. 2002). FtsZ proteins have characteristics of the cytoskeletal protein tubulin, and plant FtsZ proteins are homologues of those present in prokaryotic bacteria, which function in bacterial cell division. The FtsZ ring is stabilized by the protein ARC6, originally identified from an *Arabidopsis* mutant with few giant chloroplasts (Pyke et al. 1994; Vitha et al. 2003). FtsZ proteins assemble in the ring structure at the onset of plastid division and constriction of the ring and force generation appears to be controlled by the protein ARC5, which is a dynamin-like protein (Gao et al. 2003; Miyagishima et al. 2003) which functions on the outer surface of the plastid envelope. Evidence that ARC5 generates force and constricts the FtsZ ring complex comes from viewing isolated FtsZ rings and inducing constriction by adding ARC5 protein to them (Yoshida et al. 2006). Coordination of events on either side of the plastid envelope as the division process progresses appears to be controlled by PDV1 proteins, which form foci in the outer plastid envelope overlying the stromal FtsZ ring (Miyagishima et al. 2006). Undoubtedly, the complete plastid division machinery is a complex structure and probably contains other unknown proteins which function in a combinatorial fashion to facilitate the division process (Maple et al. 2005) especially since imaging of isolated plastid division rings containing FtsZ show distinct rings on the outer and inner surfaces of the plastid envelope (Kuroiwa et al. 2002; Miyagishima et al. 2001, 2003). Figure 3 shows a tentative model of how these proteins and the plastid division rings could be arranged. Plastid division normally occurs at the midpoint of the plastid such that the two daughter plastids, which result from the division process, are equally sized. The mechanism that ensures this equality is based on the system of Min genes, which function for a similar purpose in bacterial cell division. MinD (Colletti et al. 2000) and MinE (Itoh et al. 2001) both dictate that the FtsZ ring is allowed to form only in the middle of the plastid's long axis and is prevented from forming at either pole (Fujiwara et al. 2004). Interestingly these genes define the fact that plastids have distinct poles and are not unpolarised organelles as has been generally believed. Although the third member of the bacterial family of Min genes, MinC, appears to be absent from plant genomes, expression of the prokaryotic MinC gene in *Arabidopsis* interferes with the plastid division machinery and results in abnormally large chloroplasts (Tavva et al. 2006). Whether this is a direct interaction between the Min proteins or an effect of MinC directly on FtsZ functionality is unclear.

A clear theme which has arisen from the recent knowledge about the molecular basis of plastid division is that the division machinery currently used by chloroplasts involves proteins originally involved in prokaryotic cell division, reflecting the plastid's ancestry, and new genes which have been hijacked from the plant's genome. In addition to the genes already mentioned, *ARTEMIS* (Fulgosi et al. 2002) and *GIANT CHLOROPLAST 1* (Maple et al. 2004) are both related to prokaryotic proteins and both function in plastid division, since perturbation of them results in abnormal plastids. Conversely, ARC5 has no prokaryotic relations and ARC3 is a chimera of an FtsZ gene and a eukaryotic gene, phosphatidylinositol-4-phosphate 5-kinase (Shimada et al. 2004). It is clear that during the evolution of the plastid replication process, plant nuclear genes were recruited to interact with

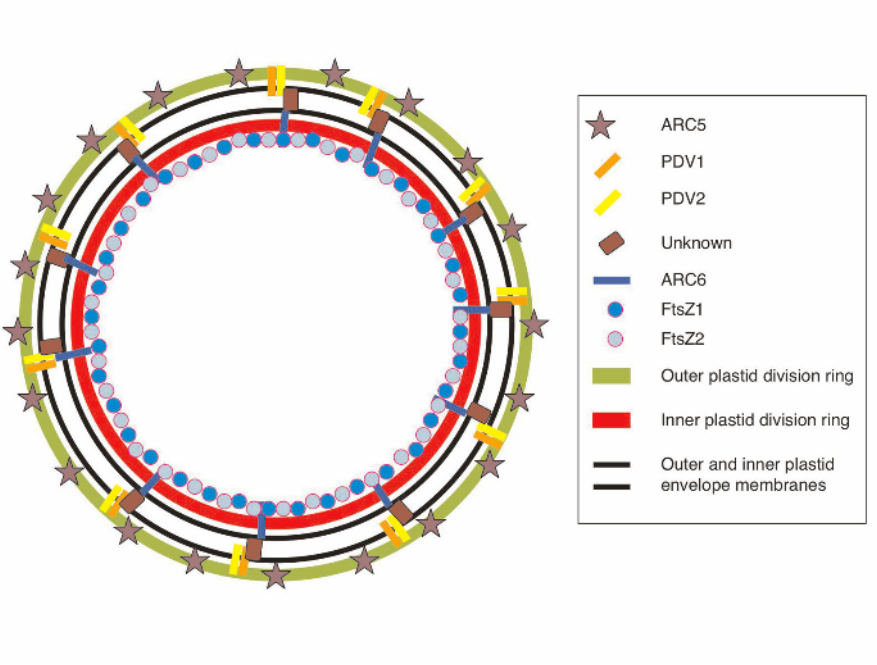

Fig. 3. A tentative model for the arrangement of proteins and the plastid dividing rings at the midpoint of a plastid about to commence division. ARC5 is associated with the outer plastid division ring and the proteins PDV1 and PDV2 link the outer plastid division ring to the plastid envelope membrane. An unknown protein spans the lumen of the envelope membrane and provides attachment points for ARC6, which links the inner envelope membrane to the inner plastid division ring and the FtsZ ring, composed of FtsZ1 and FtsZ2 proteins. Adapted from Glynn et al. (2007).

the solely prokaryotic process in order to enable control of the process in the endosymbiotic organelles by the plant nucleus. There are many questions still to be answered concerning the control of the plastid division machinery including how it is activated and stopped and how is the division of large populations of organelles during leaf cell expansion coordinated? Another often overlooked question is what suppresses the division machinery in cells where plastid replication rarely occurs and where plastid populations are relatively sparse, as in leaf epidermal cells. In addition, it is normally assumed that the binary fission type of plastid division as discussed here is the sole type of mechanism by which plastids divide. However, replication by a budding type of mechanism, which could be regarded as an extreme asymmetric type of binary division, does occur occasionally in plants (Kulandaielu and Gnanam 1985) and has been shown clearly in giant plastids of the *suffulta* mutant in tomato (Forth and Pyke 2006) where small budding vesicles bud off from the large plastid body as the chloroplasts differentiate into chromoplasts. Highly asymmetric chloroplast division has been observed in plants of *arc*11 (Marrison et al. 1999), which contain a mutation in the MinD gene (Fiji-

wara et al. 2004), so it is conceivable that a budding type mechanism could result from a breakdown in the Min centralizing system. To date, plastid division mechanisms and cell biology have only been studied in relatively few plants species and it will be interesting to ascertain the degree of variation in division mechanisms that might exist in all higher plants.

6 Stromules

Over the last decade, several important developments have occurred in our understanding of plastids. In addition to major developments in the understanding of molecular processes which occur during plastid development, a subject considered in several other chapters in this book, a renewed consideration of plastid morphology and the dynamic nature of changes in plastid morphology has also taken place. Central to this latter consideration has been the exploitation of green fluorescent protein targeted to the plastid compartment, which has revealed dramatic new aspects of plastid morphology called stromules (Fig. 1). These long thin membranous tubules containing stroma but not thylakoid membrane or chlorophyll were rediscovered in the late 1990s (Köhler et al. 1997) by imaging GFP fluorescence in plastids of tobacco and petunia containing GFP. These stromules were between 350 and 850 nm in diameter and were highly dynamic in nature extending from and retracting into the plastid body and occasionally interacting with a stromule from a neighbouring plastid. In this case, the movement of GFP from one plastid to another by stromule transfer was shown using photobleaching (Köhler et al. 2000). Ironically, the modern day observation of stromules emanating from plastids was a reconfirmation of many observations made through the last century in which microscopists have observed various protrusions and dynamic extensions of plastids in many different types of tissue (Gray et al. 2001; Kwok and Hanson 2004). Wildmann's laboratory at the University of California was famous in the 1960s for images and movies of highly dynamic plastids producing long thin extensions in the cytoplasm which can fragment, leading to the improbable suggestion that these smaller structures become mitochondria (Wildmann et al. 1962; Wildmann 1967). What we now call stromules are clearly seen in his pictures. Perhaps not surprisingly, stromule-like structures were not considered seriously within the plastid community until their rediscovery 30 years later (Tobin 1997). So how do stromules form and what do they do?

Stromules form by dynamic out growth of the plastid envelope membranes and their movement within the cytosol is controlled in part by the actin microfilament cytoskeletal system in which myosin motors link stromules and plastid bodies to the actin microfilaments (Kwok and Hanson 2003, 2004a). Careful observation of stromules with DIC optics (Gunning 2004, 2005) has revealed a great deal about the precise dynamics of stromule interaction with the microfilament tracks and clearly shows how stromules are pulled out from plastid bodies by attachment to microfilament tracks at points of attachment, not only at stromule tips but also at points along the stromule length. Sudden loss of attachment causes rapid recoil of

the stromule. In addition, stromules can also branch and rejoin forming closed loops as well as forming distinct bead-like structures along their length. Beads are particularly clear in stromules on chromoplasts in tomato fruit (Pyke and Howells 2002) although there is little evidence that such structures actually move along the stromule length. Whether the extension of stromules is entire due to pulling by the microfilament strands rather than a pushing out by a stromal pressure is unclear as is the exact source of the new membrane needed to produce a new stromule.

So what do stromules do to aid plastid function? At present the precise role of stromules is unclear but several considerations have been made. It is obvious that production of a stromule by a plastid will increase its surface area significantly and thereby increase the surface of interaction with the cytosol. Since plastids are highly active in cellular biochemistry and are sites of synthesis of many molecules important in cellular function, an increased surface area should potentially improve this interaction. This suggestion makes the assumption that the envelope membranes in the stromule have similar import capacity to that of the plastid body, a fact that has yet to be clearly addressed. The potential for movement of molecules between plastids has been demonstrated but how relevant this process might be to what actually occurs within the cell is difficult to determine. Certainly observation of plastids and stromules in the majority of cell types suggests that such joining is relatively rare and probably transitory in nature. A key point in trying to understand what stromules do is a clear distinction between their propensities in different types of cells and in particular their relative rarity in cells containing mature green chloroplasts. Thus, in mesophyll cells, which are packed with chloroplasts, stromules are rarely seen whereas in other cells containing non-green plastids such as in root cells, petal cells, epidermal cells and cultured suspension cells, stromules are much more abundant. Waters et al. (2004) showed that a decline in plastid density in the epidermal cells of expanding tobacco hypocotyls is correlated with a significant increase in stromule length raising the possibility that stromules act as a density sensing mechanism for plastids which are far apart. This could also tie in with mechanosensing proteins in the plastid envelope which sense when plastids are squashed together (Haswell and Meyerowitz 2006). In many cells containing non-green plastids, stromule networks are extensive and appear to link plastids, which are closely associated with the nucleus and surround it, to the peripheral cell membrane (Kwok and Hanson 2004b). Maybe stromules are involved with intracellular communication in some way. Fragmentation of stromules into distinct vesicles has also been suggested as a method of plastid replication since pieces of broken stromule in ripening tomato fruit cells appear to differentiate as chromoplasts. More work on stromules will be required to understand more fully these enigmatic interesting structures associated with plastids.

7 Conclusion

Our understanding of some of the cell biology aspects of plastids have improved significantly in the last two decades and the plastid has risen above the status of an

organelle that carries out only photosynthesis. The advent of omic technology has the potential for describing subtle differences between different types of plastids and may give clues as to how master controlling genes work, if they exist. Even so, we are still a long way from a clear understanding of what determines a particular plastid type in a particular type of cell and what facilitates the interconversions of different plastid types. Maybe the next decade will see big advances in addressing these questions.

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